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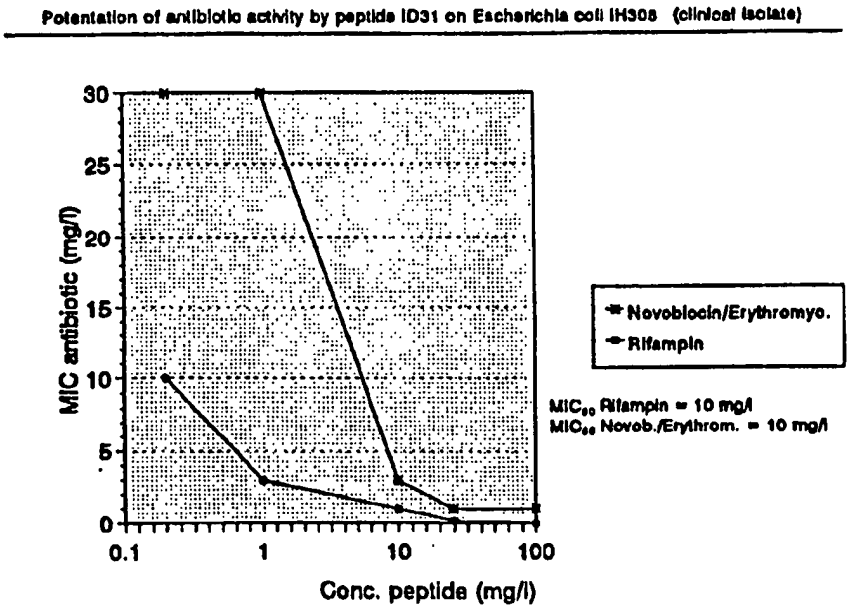

(57) Abstract

The present invention is concerned with methods of potentiating an antibiotic. The invention also includes compositions of an antibiotic and a peptide having units of the formula: (a) (A)n wherein A is Lysine or Arginine and n is an integer with a minimum value of 7; (b) (AB)m wherein A is Lysine or Arginine and B is a hydrophobic amino acid selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; m is an integer with a minimum value of 3; and (c) (ABC)p wherein A is a cationic amino acid which is Lysine or Arginine; B and C are hydrophobic amino acids which may be the same or different and are selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; p is an integer with a minimum value of 2. The compositions have potentiated antibiotic activity.

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COMPOSITIONS CONTAINING AN ANTIBIOTIC AND A PEPTIDE POTENTIATING THIS ANTIBIOTIC

FIELD OF THE INVENTION

The present invention is concerned with providing a method of potentiating antibiotics and new compositions which comprise an antibiotic and a potentiating agent which comprises a peptide which binds to lipopolysaccharide (LPS).

BACKGROUND OF THE INVENTION

Antibiotics are widely used in medicine for the treatment of infections caused by susceptible microbiological organisms. Many of these drugs have toxic side effects and/or require increased doses for the treatment of certain infections. The applicants have discovered that many different types of antibiotics, which are chemically dissimilar, may be potentiated if an effective amount of a peptide which binds to LPS is coadministered with an antibiotic to treat an infection which is caused by a susceptible organism. Certain of these peptides are disclosed in U. S. 5,371,186, which is incorporated by reference.

SUMMARY OF THE INVENTION

The applicant has discovered that antibiotics are potentiated when they are coadministered with peptides which contain the basic amino acid units (homopolymer units) as well as the basic and hydrophobic amino acids (heteropolymer units) according to the formulae: $(A)_n$, $(AB)_n$, and $(ABC)_n$ where A is any cationic amino acid (at a pH of about 7.0); B and C are any hydrophobic amino acid, both (the aliphatic cationic amino acid and the hydrophobic amino acid) that are characterized by solvent parameter values equal to or greater than +1.5kcal/mol and -1.5 kcal/mol respectively, may be coadministered with an antibiotic to potentiate

the antibiotic effect of the antibiotic. The potentiation of the antimicrobial effect of an antibiotic allows the dose of the antibiotic to be reduced while achieving the same in vivo or in vitro effect.

Accordingly, it is a primary object of the invention to provide a means of potentiating an antibiotic.

It is also an object of the invention to provide novel compositions for the treatment or prophylaxis of microbial infections.

It is also an object of the invention to provide novel methods for the treatment or prophylaxis of microbial infections which use reduced doses of antibiotic drugs.

It is also an object of this invention to provide novel compositions and methods for the treatment of microbial infections.

These and other objects of the invention will become apparent from the appended specification.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a and FIG. 1b graphically show the effect of the peptide identified herein by Sequence ID No.:31 on the potentiation of novobiocin, rifampin and a combination of novobiocin and erythromycin on various organisms.

FIG. 2a, FIG 2b, and FIG. 2c graphically show the potentiation of antibiotic activity of the peptide identified herein by Sequence ID NO.: 31 on the potentiation of novobiocin, rifampin and a combination of novobiocin and erythromycin on various organisms.

DETAILED DESCRIPTION OF THE INVENTION

The peptides of the invention have not

exhibited any growth inhibitory activity against bacteria when they have been used in the absence of an antibiotic substance. The ability of the peptides to potentiate the activity of antibiotics was therefore unexpected.

The inventors do not wish to be bound by any theory by which the invention may be explained but it is believed that the peptides of the invention interact with the membrane of pathogenic bacteria, particularly the outer membrane of gram-negative bacteria which contains LPS. The interaction of the peptide and the LPS of the bacterial outer membrane is believed to increase the permeability of the membrane to antibiotics, particularly hydrophobic/lipophilic antibiotics.

The term antibiotic is used according to Tabers Cyclopedic Medical Dictionary, 15th Ed. to describe antimicrobial substances which have the ability to inhibit the growth of or to destroy microorganisms. These substances are active in dilute solutions and may be produced in whole or in part by a microorganism or by a synthetic or semi-synthetic method.

Antibiotics which are useful in the present invention include penicillin derivatives such as penicillin G, penicillin V, penicillin G benzathine, ampicillin, amoxacillin, nafcillin, carbenicillin, dicloxacillin, bacampicillin, piperacillin, ticaricillin, mezlocillin and the like; cephalosporins such as cefazolin, cefadroxil, cephalexin, cefaclor, cefoxitin, cefonicid, ceftizoxime, cefprozil, ceftazidine, cefixime, cefpodoxime proxitel and the like; aminoglycosides such as amikacin, gentamicin, tobramycin, netilmicin, streptomycin and the like; macrolides such as erythromycin and the like; monobactams such as aztreonam and the like; rifamycin and derivatives such as rifampin, rifamide, rifaximin and the like; chloramphenicol; clindamycin; lincomycin; imipenem; vancomycin;

tetracyclines such as chlortetracycline, tetracycline, minocycline, doxycycline and the like; fusidic acid; novobiocin and the like; fosfomycin, fusidate sodium,neomycin, bacitracin, polymyxin, capreomycin, colistimethate, colistin and gramicidin.

In addition, a peptide may be used with one antibiotic or it may be used in combination with more than one antibiotic and/or in combination with other antibacterial agents. Suitable combinations include:

rifampin + erythromycin
erythromycin + sulfonamide such as sulfisoxazole
penicillin + streptomycin
rifampin + beta lattamin
rifampin + fluoroquinolones
rifampin + vancomycin
rifampin + tetracyclines
rifampin + trimetoprim
novobiocin + fluoroquinolones
trimetoprim + sulfonamides
rifampin + fusidic acid
rifampin + isoniazid
rifampin + fosfomycin
rifampin + clofazmin + dapsone
rifampin + aminoside
vancomycin + fusidic acid

Many of the antimicrobial drugs are described in Remingtons Pharmaceutical Sciences, 15th Ed., Chapter 64, which is incorporated by reference.

The peptides which are useful for potentiating the activity of antibiotics are linear or cyclic peptides having units of the formula:

(a) $(A)_n$ wherein A is Lysine or Arginine and n is an integer with a minimum value of 7;

(b) $(AB)_m$ wherein A is Lysine or Arginine and B is a hydrophobic amino acid selected from the group consisting

of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; m is an integer with a minimum value of 3; and (c) $(ABC)_p$ wherein A is a cationic amino acid which is Lysine or Arginine; B and C are hydrophobic amino acids which may be the same or different and are selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; p is an integer with a minimum value of 2. The peptides of the invention may be terminated independently with a hydrogen atom or any of the naturally occurring amino acids, a fatty acid residue or a carbohydrate residue. In addition the retroinverted peptides of the peptides described herein may also be employed.

The preferred peptides for use in the invention will also have a ratio of aliphatic cationic amino acids to hydrophobic amino acids ($R_{c/h}$) of at least 0.5 and within the range of about 0.5 to 10.0 which is computed by using the solvent parameter values only for those amino acids which are present in the peptides which have a solvent parameter value equal to or greater than +1.5kcal/mol (lysine and arginine) and -1.5kcal/mol (valine, isoleucine,leucine, tryrosine, phenylalanine and tryptophane) as measured according to Levitt, J. Mol. Biol. 104,59 (1976), which is incorporated by reference.

The minimal effective peptide sequence for use in potentiating an antibiotic comprises six to seven amino acid residues containing a minimum of three aliphatic cationic amino acids, with a ratio of aliphatic cationic amino acids to hydrophobic amino acids of equal to or greater than 0.5 ($R_{c/h}$ wherein c is the number of cationic amino acids in the peptide and h is the number of hydrophobic amino acids in the peptide). This ratio is believed to be the minimum although sequences of ten amino acids with a ratio ($R_{c/h}$) equal to or greater than 1.0 are optimal for expression of biological activity.

The peptide units which are represented by formula (a), (b) and (c) represent discrete peptides which will potentiate antibiotics have specific formulas which are identical with the units of formula (a), (b) and (c) as well as peptides which will bind endotoxin in the LAL inhibition test and which include as a part of their structure units of formula (a), (b) and (c), in addition to other amino acids, are included within the peptides which comprise the invention.

The peptides should not exhibit hemolytic activity when equal volumes of a solution of the peptide in isotonic saline, at a minimum peptide concentration of 0.1mg/ml and a solution of 10%w/w fresh human erythrocytes in isotonic saline are incubated at 37°C. for 30 minutes and no rupture of the erythrocytes and release of hemoglobin is detected visually or by use of a spectrophotometer (540nm).

The minimum values for n, m and p have been determined experimentally on the basis of the observation that when the peptide is linear, it will have at least 7 amino acid units and when said peptide is cyclic or a polymer having several cycles, i.e. 2 to 6 cycles, it will have a ring structure that has a minimum of 6 amino acid units and preferably a maximum of 7 amino acid units; said peptides having a ratio of aliphatic cationic amino acids to hydrophobic amino acids which is equal to or greater than 0.5.

When the peptides are of the formula $(A)_n$, $(AB)_m$ or $(ABC)_p$, i.e. when these formulas do not represent units of a larger peptides, n will be from 7 to 500 and preferably from 7 to 10; m will be from 3 to 200 and preferably from 4 to 20 and p will be from 2 to 100 and preferably from 4 to 20.

Examples of the peptides are listed below. Those peptides which are not novel are marked by an

asterisk:

$(Lys)_{10}$ (SEQ ID NO: 1);

$(Lys)_{30}$* (SEQ ID NO: 2);

$(Lys)_{434}$* (SEQ ID NO: 3);

$(Lys\text{-}Asp)_5$ (SEQ ID NO: 4);

$(Lys\text{-}Phe)_5$ (SEQ ID NO: 5);

Lys-Phe-Leu-Lys-Lys-Thr-Leu (SEQ ID NO: 6);

$(Lys\text{-}Phe\text{-}Leu)_2$-Lys (SEQ ID NO: 7);

$(Lys\text{-}Phe\text{-}Leu)_3$-Lys (SEQ ID NO: 8);

$(Arg\text{-}Tyr\text{-}Val)_3$ (SEQ ID NO: 9);

$(Lys\text{-}Phe\text{-}Phe)_3$-Lys (Seq ID NO: 10);

$(Lys\text{-}Leu\text{-}Leu)_3$ (SEQ ID NO: 11);

$(Lys)_6(Phe\text{-}Lys)_2$ (SEQ ID NO: 12);

Cys-$(Lys)_5$-Cys
s----------s (SEQ ID NO: 13);

Cys-Lys-Phe-Lys-Lys-Cys
s----------s (SEQ ID NO: 14);

Lys-Phe-Lys-Cys-Lys-Phe-Lys-Phe-Lys-Cys
s----------s (SEQ ID NO: 15);

Lys-Leu-Lys-Cys-Lys-Leu-Lys-Leu-Lys-Cys
s----------s (SEQ ID NO: 16);

Arg-Thr-Arg-Cys-Arg-Phe-Lys-Arg-Arg-Cys
s----------s (SEQ ID NO: 17);

Lys-Cys-$(Lys\text{-}Phe\text{-}Lys)_2$-Cys-Lys
s----------s (SEQ ID NO: 18);

Cys-$(Lys)_4$-$(Phe)_4$-Cys
s----------s (SEQ ID NO: 19);

Cys-$(Lys\text{-}Phe\text{-}Leu)_3$-Lys-Cys
s----------s (SEQ ID NO: 20);

Val-Lys-Ala-Leu-Arg-Val-Arg-Arg-Leu (SEQ ID NO: 21);

Lys-Ser-Leu-Ser-Leu-Lys-Arg-Leu-Thr-Tyr-Arg (SEQ ID NO:22);

Lys-Val-Arg-Lys-Ser-Phe-Phe-Lys-Val (SEQ ID NO: 23);

Phe-Leu-Lys-Pro-Gly-Lys-Val-Lys-Val (SEQ ID NO: 24);

Lys-Glu-Leu-Lys-Arg-Ile-Lys-Ile (SEQ ID NO: 25);

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu (SEQ ID NO: 26);

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu-Lys (SEQ ID NO: 27);

Lys-Arg-Leu-Lys-Trp-Lys-Tyr-Lys-Gly-Lys-Phe (SEQ

ID NO: 28); and

Cys-Gln-Ser-Trp-Lys-Ser-Ser-Glu-Ile-Arg-Cys-Gly-Lys
s----------s (SEQ ID NO: 29).

Cys-Lys-Phe-Leu-Lys-Lys-Cys
s - - - - - - - - - - - - s (SEQ ID NO:30)

Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys(SEQ ID NO:31)
s - - - - - - - - - - s

Lys-Phe-Leu-Lys-Lys-Thr(SEQ ID NO: 32)

Cys-Lys-Lys-Leu-Phe-Lys-Cys-Lys-Thr-Lys
s - - - - - - - - - - - - s(SEQ ID NO: 33)

Cys-Lys-Lys-Leu-Phe-Lys-Cys-Lys-Thr
s - - - - - - - - - - - - -s(SEQ ID NO: 34)

Ile-Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys
s - - - - - - - - - - - - s(SEQ ID NO: 35)

Ile-Lys-Thr-Lys-Lys-Phe-Leu-Lys-Lys-Thr(SEQ ID NO: 36)

Ile-Lys-Phe-Leu-Lys-Phe-Leu-Lys-Phe-Leu-Lys(SEQ ID NO: 37)

Lys-Phe-Leu-Lys-Phe-Leu-Lys(SEQ ID NO: 38)

Arg-Tyr-Val-Arg-Tyr-Val-Arg-Tyr-Val(SEQ ID NO: 39)

Lys-Phe-Phe-Lys-Phe-Phe-Lys-Phe-Phe(SEQ ID NO: 40)

Ile-Lys-Phe-Leu-Lys-Phe-Leu-Lys-Phe-Leu(SEQ ID NO:41)

$(Lys)^6$Phe-Leu-Phe-Leu(SEQ ID NO:42)

Cys-Lys-Phe-Lys-Phe-Lys-Phe-Lys-Phe-Cys
s----------s(SEQ ID NO: 43

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu-Lys(SEQ ID NO: 44)

Lys-Arg-Leu-Lys-Trp-Lys-Tyr-Lys-Gly-Lys-Phe(SEQ ID NO: 45)

The peptides for use in the present invention may be synthesized by classical methods of peptide chemistry using manual or automated techniques as well as by DNA recombinant technology. The synthetic procedure

comprises solid phase synthesis by Fmoc chemistry, cleavage (TFA 95%+Et-$(SH)_2$ 5%), followed by vacuum evaporation. Thereafter, the product is dissolved in 10% acetic acid, extracted with ether, concentrated at 0.1 mg/ml at pH of 6.0-7.5. Stirring under filtered air followed for 1 to 6 hours in case of the Cysteine-containing peptides and finally desalting by reverse phase chromatography is carried out.

A particular automated method of preparing peptides for use in the present invention is based on the use of an automatic synthesizer (Milligen Mod.9050 (MILLIPORE, Burlington, MA) on a solid phase support of polyamide/Kieselguhr resin (2.0g). The amino acids used in the synthesis of the peptide analogs are Fmoc-aa-Opfp derivatives (9-Fluorenylmethylcarbonyl-aa-O-pentafluorophenyl ester) of each amino acid(aa) involved in the considerd sequences using 0.8 mol of each amino acid to sequentially form the peptide.

Each cycle of synthesis may be performed at room temperature (20°C) and involves the following steps of reaction:

Step 1 - Deprotection

The first aa Fmoc-protected at the amino group, was treated with a 20% solution of piperidine for 7 minutes in order to remove the Fmoc alpha-protecting group. Washing with dimethylformamide followed for 12 minutes to remove all traces of piperidine. Deprotection and washing were run continuously through the column containing the resin by means of a pump at a flow of 5ml/min.

Step 2 - Activation of the Fmoc-aa-Opfp derivative

The amino and carboxy-protected amino acid due, according to the desired sequence, was activated after its dissolution in 5 ml of dimethylformamide, by a catalytic amount of hydroxybenzotriazol (0.5 ml of a 5% w/v

solution in dimethylformamide).

Step 3 - Acylation

The activated and protected Fmoc-aa-Opfp derivative was then recycled for 30 minutes through the column by the pump at 5ml/min in order to obtain coup[ling of the introduced aa at the alpha-amino group (previously deprotected as reported in Step 1) of the amino acid preceding the new one in the desired sequence.

Step 4 - Washing

Washing of the matrix in the column followed by dimethylformamide for 2 minutes at 5 ml/min before a new cycle began.

At the completion of the synthesis, the peptide on the resin support was cleaved by 95% Trifluoroacetic acid (TFA) with 5% Ethane dithiol as a scavenger, if Cysteine residues were present in the aa sequence, at room temperature for 2 hours. After separation of the cleaved peptide from the resin by filtration, the solution was concentrated by vacuum evaporation to dryness. The collected solid residue was then solubilized in 10% acetic acid at a concentration of 10-20 mg/ml and several extractions by diethyl ether followed (six to eight extractions with half the volume of the peptide solution) in order to remove the scavenger Ethane dithiol. The peptide solution was then neutralized by 0.1 N ammonium hydroxide and adjusted to the concentration of roughly 0.1 mg/ml. The solution was then stirred under air for 1 to 6 hours in order to obtain the selective oxidation of the two sulfhydryl groups belonging to the Cys residues of the sequence. In this way, only monomeric oxidized peptides were obtained with no traces of polymeric material. The solution of oxidized peptide was then desalted by reverse-phase chromatography on SEP-PAK C-18 cartridges (MILLIPORE) and finally freeze dried. The products were analyzed by

high-performance liquid chromatography (HPLC) analysis as well as by chemical analysis of the synthetic structures.

Fast atom bombardment may be used to confirm the calculated mass of the peptides.

The peptides described herein which exhibit the absence or a low level of hemolysis may be used in the treatment of infections in mammals including humans at doses of about 0.1mg-2.0mg/kg of body weight or may be used at a level of about 0.2mg to about 1.0mg/kg of body weight and the amount may be administered in divided doses on daily basis prior to, simultaneously with or after the administration of an antibiotic. Generally the doses of the antibiotic will be reduced by from about 90% to about 10% of the standard therapeutic dose of a given antibiotic as shown in standard compendia such as the 1994 Physicians Desk Reference, which is incorporated by reference. The combination of the peptide and the antibiotic may be administered prophylactically to patients who may be exposed to or have been exposed to organisms which may cause infection. The particular dose of a particular peptide with a particular antibiotic may be varied within or without the range that is specified herein depending on the particular application or severity of the infection and the condition of the host. Those who are skilled in the art may ascertain the proper dose using standard procedures. A convenient dose of a combined formulation of the peptide and the antibiotic may be 0.1-1.0mg/Kg of body weight of peptide with 0.25-40mg/Kg of body weight of antibiotic administered daily in single or multiple doses in order to achieve and maintain therapeutic plasma concentrations.

The peptides may be administered intravenously and parenterally using well known pharmaceutical carriers or inert diluents and the antibiotics may be administered intravenously, parenterally or orally depending on the

particular antibiotic. Aqueous, physiologically compatible diluents are preferred. A composition containing both the peptide and the antibiotic may be placed in the same sterile container for dilution with a suitable diluent such as sterile isotonic saline or sterile water for injection prior to administration. If the peptide and the antibiotic are not compatible, they may be placed in containers that provide a means for separation of the components until just prior to use or they may be placed in separate containers. The invention also includes topical preparations containing the peptide and antibiotic in the form of ophthalmic ointments or drops; otological preparations such as viscous liquids e.g. propylene glycol based sterile solutions or dispersions; and topical creams and ointments for the treatment and/or prevention of skin infections. Suitable vehicles and the techniques for preparing suitable vehicles are set forth in Remingtons Pharmaceutical Sciences, 17th Ed., Mack Pub. Co., Easton, PA 18042, Chapters 84, 87 and 88, which is incorporated by reference. Generally the concentration of the peptide and the antibiotic in these preparations will be sufficient to exert an antimicrobial effect. These amounts will vary depending on the particular drugs which are selected and may be determined by routine experimentation. Generally the peptides may be used at a concentration of 0.1-5wt% and the antibiotics may be used at from 90% to 10% of the usual therapeutic amount.

When other antibacterial agents are used in combination with an antibiotic and the peptide composition, the total amount of the antibacterial may also be reduced from 10 to 90% while still obtaining an enhanced therapeutic response with reduced toxicity.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Example 1

The growth inhibition of the combination of a peptide and an antibiotic was demonstrated in vitro using microdilution plates, the checkerboard technique and a bacterial inoculum size of 10^4 bacterial cells/ml. The general assay medium was L broth (pH7.2) which contained 10g of tryptone (Difco Laboratories, Detroit, MI), 5g of yeast extract (Oxoid Ltd., Hampshire, UK) and 5g of sodium chloride per liter. After an incubation time of 18 hours at 37°C, the growth of each microtiter well is measured with a Titerteck Multiscan spectrophotometer at 405nm. Before reading, the spectrophotometer was blanked with corresponding uninoculated drug-containing media. The minimum inhibitory concentration (MIC) of an antibiotic was defined as the lowest concentration of the antibiotic expressed in mg/l which reduced the growth of the target bacteria by ≥90% (MIC_{90}).

The results of the MIC tests show that the combination of an antibiotic and a peptide provides synergistic growth inhibition activity. These results are summarized in Table I and are shown specifically for a representative peptide in FIG. 1a,1b and FIG 2a, 2b, 2c.

TABLE I

Peptide Seq. ID	Concentration of peptide (mg/1)	E. coli IH3080 Experiment I MIC Rifampin	E. coli IH3080 Experiment II MIC Fusidic a.
None	0	10	300
30	1	10	300
	10	3	300
	100	0.1	10
31	1	3	300
	10	1	100
	100	0.1	10
35	1	10	300

	10	3	100
	100	1	100
40	1	1	100
	10	0.03	1
41	1	1	100
	10	0.03	1
	30	0.01	1

Peptide Seq, ID	Concentration of peptide (mg/l)	E. coli IH3080 Experiment III MIC Novobiocin	E. coli IH3080 Experiment IV MIC Erythrom.
None	0	30	30
30	1	30	30
	10	10	30
	100	1	1
31	1	30	30
	10	3	10
	100	1	1
35	1	30	30
	10	30	30
	100	3	30
40	1	1	30
	10	1	3
	30	1	1
41	1	10	10
	10	1	1

Peptide Seq. ID	Concentration of peptide (mg/l)	E. coli IH3080 Experiment I MIC Rifampin	E. coli IH3080 Experiment II MIC Fusidic a.
42	1	10	300
	10	3	300
43	1	10	300
	10	1	100
	30	0.1	10
26	1	10	300
	10	1	300
	100	0.3	30
28	1	1	300
	10	0.3	100
	100	0.01	1

Peptide Seq. ID	Concentration of peptide (mg/l)	E. coli IH3080 Experiment III MIC Novobiocin	E. coli IH3080 Experiment IV MIC Erythrom.
42	1	10	100
	10	10	30
43	1	30	100
	10	10	30
	30	1	1
26	1	30	30
	10	10	30
	100	3	10
28	1	10	30
	10	3	10
	100	1	1

Peptide Seq. ID	Concentration of peptide (mg/l)	S.typhi SH5014 Experiment I MIC Rifampin	S. typhi SH5014 Experiment II MIC Fusidic a.
None	0	10	>300
30	1	10	>300
	10	10	>300
	100	0.1	30
31	1	10	>300
	10	10	>300
	100	0.03	3
35	1	10	>300
	10	10	>300
	100	3	300
40	1	3	100
	10	0.01	1
41	1	3	100
	3	0.01	1

Peptide Seq. ID	Concentration of Peptide (mg/ml)	S.typhi SH5014 Experiment III MIC Novobiocin	S.typhi SH5014 Experiment IV MIC Erythromycin.
None	0	30	100
30	1	30	100
	10	30	100
	100	1	10
31	1	10	100

	10	10	100
	100	1	3
35	1	30	100
	10	10	100
	100	10	100
40	1	10	100
	10	1	1
41	1	10	100
	3	1	1

Peptide Seq. ID	Concentration of peptide (mg/l)	S.typhi SH5014 Experiment I MIC Rifampin	S.typhi SH5014 Experiment II MIC Fusidic a.
42	1	10	>300
	10	10	300
43	1	10	>300
	10	3	300
	30	0.1	1
26	1	10	>300
	10	3	100
	100	0.3	30
28	1	10	300
	10	1	300
	30	0.1	100

Peptide Seq. ID	Concentration of peptide (mg/l)	S.typhi. SH5014 Experiment III MIC Novobiocin	S.typhi. SH5014 Experiment IV MIC Erythrom.
42	1	30	100
	10	10	100
43	1	30	100
	10	10	100
	30	1	1
26	1	30	100
	10	10	100
	100	3	30
28	1	10	100
	10	3	100
	30	1	10

Peptide Seq. ID	Concentration of peptide (mg/l)	Ps. aeroginosa. PAO1 Experiment I MIC Rifampin	Ps. aeroginosa. PAO1 Experiment II MIC Fusidic a.
None	0	>10	>300
30	1	>10	>300
	10	>10	>300
	100	10	>300
31	1	>10	>300
	10	10	>300
	100	3	300

Peptide Seq. ID	Concentration of peptide (mg/l)	Ps. aeroginosa PAO1 Experiment III MIC Novobiocin	Ps. aeroginosa. PAO1 Experiment IV MIC Erythrom.
None	0	>30	100
30	1	>30	100
	10	>30	100
	100	>30	100
31	1	>30	100
	10	>30	100
	100	>30	100

Peptide Seq. ID	Concentration of peptide (mg/l)	Kl.pneumoniae 12854 Experiment I MIC Rifampin	Kl.pneumoniae 12854 Experiment II MIC Fusidic a.
None	0	10	>300
30	1	10	>300
	10	10	>300
	100	1	100
31	1	10	>300
	10	10	300
	100	1	30
35	1	10	>300
	10	10	>300
	100	10	300
40	1	10	100
	10	0.1	10
	100	0.01	1
41	1	3	300
	10	0.03	3
	30	0.01	1

Peptide Seq. ID	Concentration of peptide (mg/l)	Kl.pneumoniae 12854 Experiment III MIC Novobiocin	Kl.pneumoniae 12854 Experiment IV MIC Erythrom.
None	0	30	>100
30	1	30	>100
	10	30	>100
	100	10	30
31	1	30	>100
	10	30	>100
	100	3	30
35	1	30	>100
	10	30	>100
	100	10	>100
40	1	30	>100
	10	3	3
	100	1	1
41	1	10	>100
	10	1	3
	30	1	1

Peptide Seq. ID	Concentration of peptide (mg/l)	Kl.pneumoniae 12854 Experiment I MIC Rifampin	Kl. pneumoniae 12854 Experiment II MIC Fusidic a.
42	1	10	>300
	10	10	>300
	100	1	100
43	1	10	>300
	10	10	300
	100	0.3	30
26	1	10	>300
	10	10	300
	100	3	100
28	1	10	>300
	10	10	300
	100	1	30

Peptide Seq. ID	Concentration of peptide (mg/l)	Kl. pneumoniae 12584 Experiment III MIC Novobiocin	Kl. pneumoniae 12584 Experiment IV MIC Erythromycin
42	1	30	>100
	10	30	>100
	100	1	10
43	1	30	>100
	10	30	>100
	100	3	10
26	1	30	>100
	10	30	>100
	100	10	100
28	1	30	>100
	10	10	100
	100	3	30

Peptide Seq. ID	Concentration of peptide (mg/l)	E. cloa 12645 Experiment I MIC Rifampin	E. cloa 12645 Experiment II MIC Fusidic a.
None	0	10	>300
30	1	10	>300
	10	10	>300
	100	0.3	30
31	1	10	>300
	10	10	300
	100	1	30
35	1	10	>300
	10	10	>300
	100	3	300
40	1	3	100
	10	0.1	3
	100	0.01	1
41	1	3	100
	10	0.03	1
	30	0.01	1

Peptide Seq. ID	Concentration of Peptide (mg/l)	E. cloa 12645 Experiment III MIC Novobiocin	E. cloa. 12645 Experiment IV MIC Erythrom.
None	0	>30	>100
30	1	>30	>100
	10	>30	>100
	100	10	30
31	1	>30	>100
	10	>30	>100
	100	10	100
35	1	>30	>100
	10	>30	>100
	100	30	>100
40	1	10	100
	10	1	1
	100	1	1
41	1	30	>100
	10	1	1
	30	1	1

Peptide Seq. ID	Concentration of peptide (mg/l)	E. cloa 12645 Experiment I MIC Rifampin.	E. cloa 12645 Experiment II MIC Fusidic a.
42	1	10	>300
	10	10	>300
	100	0.01	30
43	1	10	>300
	10	3	>300
	100	0.3	30
26	1	10	>300
	10	10	>300
	100	1	100
28	1	10	>300
	10	3	300
	100	1	100

Peptide Seq. ID	Concentration of peptide (mg/1)	E. cloa. 12645 Experiment III MIC Novobiocin	E.cloa. 12645 Experiment IV MIC Erythrom.
42	1	>30	>100
	10	>30	>100
	100	3	10
43	1	>30	>100
	10	>30	100
	100	10	10
26	1	>30	>100
	10	30	>100
	100	10	100
28	1	>30	>100
	10	30	100
	100	10	100

The data in Table II shows that the peptides when used alone have no significant antibacterial activity. Thes data were obtained using the general procedure set forth above:

TABLE II

	Peptide SEQ ID NO:				
Bacterial strain	30	31	35	40	41
E. coli 1H3080	>100	>100	>100	100	30
S. Typhimurium SH5014	>100	>100	>100	30	30
Klebs pneum. 12854	>100	>100	>100	>100	100
Enterob. cloacae 12654	>100	>100	>100	>100	100
Pseud. aeroginosa PAO1	>100	>100	>100	30	30
E. coli SM 101	>100	>100	>100	30	10
Micrococcus luteus ML36	100	100	>100	10	30

	Peptide SEQ ID NO:			
	42	43	26	28
E. coli 1H3080	30	100	>100	>100
S. Typhimurium SH5014	30	100	>100	100

Klebs pneum. 12854	>100	>100	>100	>100
Enterob. cloacae 12654	>100	>100	>100	>100
Pseud. aeroginosa PAO1	>100	>100	>100	>100
E. coli SM 101	30	30	>100	100
Micrococcus luteus ML36	10	30	>100	30

Example

A human patient suffering from an infection caused by K. pneumoniae may be treated with a combination of Rifampin (0.5mg/Kg of body weight/IV every 8 hours in normal saline) and Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys
s----------s
(SEQ ID No.:31) (1mg/Kg of body weight/IV every 8 hours in normal saline). The dose of Rifampin is 10 to 20% by weight of the usual clinical dose of Rifampin which is administered as the sole therapeutic agent. This reduces the possibility of any toxic side effects of Rifampin without reduction of the clinical efficacy of Rifampin.

CLAIMS

1. A method for the potentiation of the activity of an antibiotic which comprises coadministering an antibiotic and a peptide which contains the structural amino acid units as well as the basic and hydrophobic amino acids according to the formulae: $(A)_n$, $(AB)_n$, and $(ABC)_n$ where A is any aliphatic cationic amino acid (at a pH of about 7.0); B and C are any hydrophobic amino acid.

2. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is a linear or cyclic peptides having units of the formula:
(a) $(A)_n$ wherein A is Lysine or Arginine and n is an integer with a value of 7 to 10;
(b) $(AB)_m$ wherein A is Lysine or Arginine and B is a hydrophobic amino acid selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; m is an integer with a minimum value of 3; and
(c) $(ABC)_p$ wherein A is a cationic amino acid which is Lysine or Arginine; B and C are hydrophobic amino acids which may be the same or different and are selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; p is an integer with a minimum value of 2.

3. A method as defined in claim 1 where the antibiotic is selected from the group consisting of penicillin derivatives; cephalosporins; aminoglycosides; erythromycin; monobactams; rifamycin and derivatives thereof; chloramphenicol; clindamycin; lincomycin; imipenem; vancomycin; tetracyclines; fusidic acid and novobiocin.

4. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide has units of the formula $(A)_n$ where n has a value of 7 to 10.

5. A method for the potentiation of the activity of an

antibiotic as defined in claim 1 wherein the peptide has units of the formula $(AB)_m$.

6. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide has units of the formula $(ABC)_p$.

7. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys)_{10}$. (SEQ ID NO: 1)

8. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys\text{-}Glu)_5$. (SEQ ID NO: 4)

9. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys\text{-}Phe)_5$. (SEQ ID NO: 5)

10. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Phe-Leu-Lys-Lys-Thr-Leu. (SEQ ID NO: 6)

11. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein in which the peptide is of the formula:

$(Lys\text{-}Phe\text{-}Leu)_2\text{-}Lys$. (SEQ ID NO: 7)

12. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys\text{-}Phe\text{-}Leu)_3\text{-}Lys$. (SEQ ID NO: 8)

13. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Arg\text{-}Tyr\text{-}Val)_3$. (SEQ ID NO: 9)

14. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of

the formula:

$(Lys\text{-}Phe\text{-}Phe)_3\text{-}Lys$. (Seq ID NO: 10)

15. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys\text{-}Leu\text{-}Leu)_3$ (SEQ ID NO: 11)

16. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys)_6(Phe\text{-}Lys)_2$. (SEQ ID NO: 12)

17. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-$(Lys)_5$-Cys
s----------s. (SEQ ID NO: 13)

18. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Lys-Phe-Lys-Lys-Cys
s--------------------s. (SEQ ID NO: 14)

19. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Phe-Lys-Cys-Lys-Phe-Lys-Phe-Lys-Cys
s----------s. (SEQ ID NO: 15)

20. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Leu-Lys-Cys-Lys-Leu-Lys-Leu-Lys-Cys
s----------s. (SEQ ID NO: 16)

21. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Arg-Thr-Arg-Cys-Arg-Phe-Lys-Arg-Arg-Cys
s----------s. (SEQ ID NO: 17)

22. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of

the formula:

Lys-Cys-$(Lys-Phe-Lys)_2$-Cys-Lys
s----------s. (SEQ ID NO: 18)

23. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-$(Lys)_4$-$(Phe)_4$-Cys
s----------s. (SEQ ID NO: 19)

24. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-$(Lys-Phe-Leu)_3$-Lys-Cys
s----------s. (SEQ ID NO: 20)

25. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula: Val-Lys-Ala-Leu-Arg-Val-Arg-Arg-Leu (SEQ ID NO: 21)

26. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Ser-Leu-Ser-Leu-Lys-Arg-Leu-Thr-Tyr-Arg (SEQ ID NO:22)

27. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Val-Arg-Lys-Ser-Phe-Phe-Lys-Val (SEQ ID NO: 23)

28. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Phe-Leu-Lys-Pro-Gly-Lys-Val-Lys-Val (SEQ ID NO: 24)

29. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Asp-Leu-Lys-Arg-Ile-Lys-Ile (SEQ ID NO: 25)

30. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu (SEQ ID NO: 26)

31. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu-Lys (SEQ ID NO: 27)

32. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Arg-Leu-Lys-Trp-Lys-Tyr-Lys-Gly-Lys-Phe (SEQ ID NO:28)

33. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Gln-Trp-Lys-Ser-Ser-Asp-Ile-Arg-Cys-Gly-Lys
s----------s (SEQ ID NO: 29)

34. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Lys-Phe-Leu-Lys-Lys-Cys
s----------s (Seq ID NO: 30)

35. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys(SEQ ID NO:31)
s - - - - - - - - - - s

36. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Phe-Leu-Lys-Lys-Thr(SEQ ID NO: 32)

37. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Lys-Lys-Leu-Phe-Lys-Cys-Lys-Thr-Lys
s - - - - - - - - - - - - s(SEQ ID NO: 33)

38. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Lys-Lys-Leu-Phe-Lys-Cys-Lys-Thr
s - - - - - - - - - - - - s(SEQ ID NO: 34)

39. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Ile-Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys
s - - - - - - - - - - - - s(SEQ ID NO: 35)

40. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Ile-Lys-Thr-Lys-Lys-Phe-Leu-Lys-Lys-Thr(SEQ ID NO: 36)

41. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Ile-Lys-Phe-Leu-Lys-Phe-Leu-Lys-Phe-Leu-Lys(SEQ ID NO: 37)

42. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Phe-Leu-Lys-Phe-Leu-Lys(SEQ ID NO: 38)

43. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Arg-Tyr-Val-Arg-Tyr-Val-Arg-Tyr-Val(SEQ ID NO: 39)

44. A method for the potentiation of the activity of an

antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Phe-Phe-Lys-Phe-Phe-Lys-Phe-Cys(SEQ ID NO: 40)

45. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Ile-Lys-Phe-Leu-Lys-Phe-Leu-Lys-Phe-Leu(SEQ ID NO:41)

46. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

$(Lys)_6$Phe-Leu-Phe-Leu(SEQ ID NO:42)

47. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Cys-Lys-Phe-Lys-Phe-Lys-Phe-Lys-Phe-Cys
s----------s(SEQ ID NO: 43

48. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu-Lys(SEQ ID NO: 44)

49. A method for the potentiation of the activity of an antibiotic as defined in claim 1 wherein the peptide is of the formula:

Lys-Arg-Leu-Lys-Trp-Lys-Tyr-Lys-Gly-Lys-Phe(SEQ ID NO: 45)

50. A composition for the potentiation of the activity of an antibiotic which comprises an antibiotic and a peptide which contains the structural amino acid units as well as the basic and hydrophobic amino acids according to the formulae: $(A)_n$, $(AB)_n$, and $(ABC)_n$ where A is any aliphatic cationic

amino acid (at a pH of about 7.0); B and C are any hydrophobic amino acid.

51. A pharmaceutical composition which comprises an antibacterial effective amount of an antibiotic and a antibiotic potentiating effective amount of a peptide of the formula:
(a) $(A)_n$ wherein A is Lysine or Arginine and n is an integer with a minimum value of 7.
(b) $(AB)_m$ wherein A is Lysine or Arginine and B is a hydrophobic amino acid selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; m is an integer with a minimum value of 3; and
(c) $(ABC)_p$ wherein A is a cationic amino acid which is Lysine or Arginine; B and C are hydrophobic amino acids which may be the same or different and are selected from the group consisting of Valine, Leucine, Isoleucine, Tyrosine, Phenylalanine and Tryptophan; p is an integer with a minimum value of 2 and a pharmaceutical carrier.

52. A method of treating a bacterial infection which comprises administering to a host an antibacterial effective amount of an antibiotic and an antibiotic potentiating effective amount of a peptide of claim 1 which does not exhibit hemolytic activity when tested by combining equal volumes of a solution of the peptide in isotonic saline, at a minimum concentration of 0.1mg/ml and a solution of 10%w/w fresh human erythrocytes in isotonic saline are incubated at 37°C.for 30 minutes and no rupture of the erythrocytes and release of hemoglobin is detected visually or by use of a spectrophotometer (540nm).

53. A pharmaceutical composition as defined in claim 50 wherein the antibiotic is selected from the group consisting of penicillin derivatives; cephalosporins; aminoglycosides;

erythromycin; monobactams; rifamycin and derivatives thereof; chloramphenicol; clindamycin; lincomycin; imipenem; vancomycin; tetracyclines; fusidic acid and novobiocin.

54. A method for the potentiation of of the activity of an antibiotic which comprises coadministering an antibiotic and a peptide which contains amino acid sequences in which the amino acids are inverted with respect to their original position in the sequence of the peptides of claim 1.

55. A pharmaceutical composition which comprises an antibacterial effective amount of an antibiotic and an antibiotic potentiating effective amount of a peptide which contains amino acid sequences in which the amino acids are inverted with respect to their original position in the sequence of the peptides of claim 1.

56. A method for the potentiation of the activity of an antibiotic which comprises coadministering an antibiotic and a peptide which exhibits no direct antimicrobial activity and no detectable hemolytic activity on human erythrocytes at a concentration of 0.1mg of peptide/ml of an aqueous diluent.

57. A pharmaceutical composition which comprises an antibacterial effective amount of an antibiotic and an antibiotic potentiating effective amount of a peptide which shows no direct antimicrobial activity on human erythrocytes when tested by combining equal volumes of a solution of the peptide in isotonic saline, at a minimum concentration of 0.1mg/ml and a solution of 10%w/w fresh human erythrocytes in isotonic saline are incubated at 37°C.for 30 minutes and no rupture of the erythrocytes and release of hemoglobin is detected visually or by use of a spectrophotometer (540nm).

Fig.1a

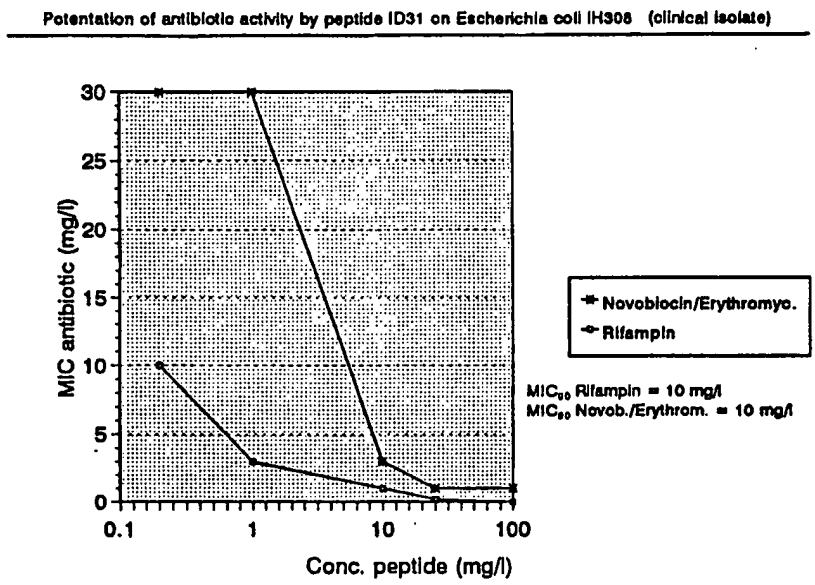

Potentation of antibiotic activity by peptide ID31 on Escherichia coli IH308 (clinical isolate)
MIC antibiotic (mg/l)
30
25
20
15
10
5
0
0.1
1
10
100
Conc. peptide (mg/l)
Novobiocin/Erythromyc.
Rifampin
MIC90 Rifampin = 10 mg/l
MIC90 Novob./Erythrom. = 10 mg/l

Fig.1b

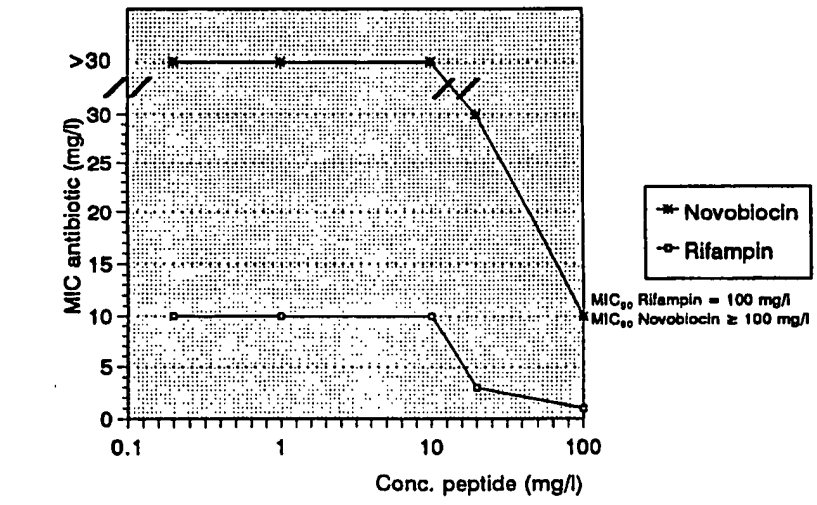

Potentation of antibiotic activity by peptide ID31 on Enterobacter cloacae 12645 (clinical isolate)
MIC antibiotic (mg/l)
>30
30
25
20
15
10
5
0
0.1
1
10
100
Conc. peptide (mg/l)
Novobiocin
Rifampin
MIC90 Rifampin = 100 mg/l
MIC90 Novobiocin ≥ 100 mg/l

Fig.2a

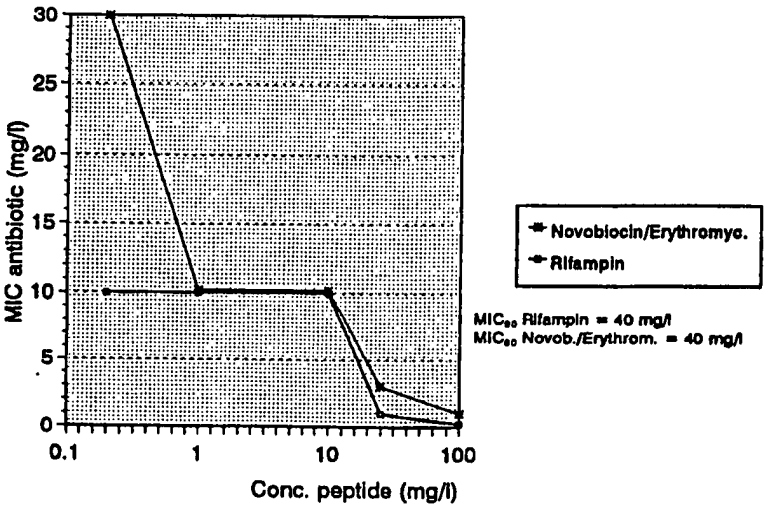


Fig.2b

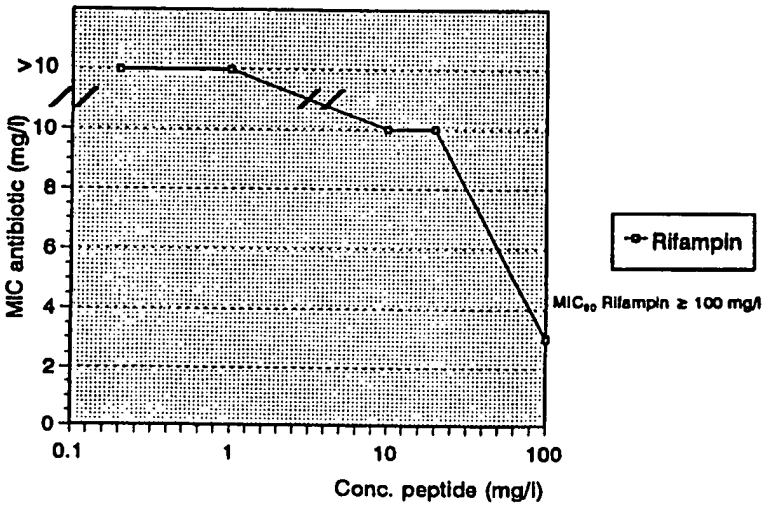
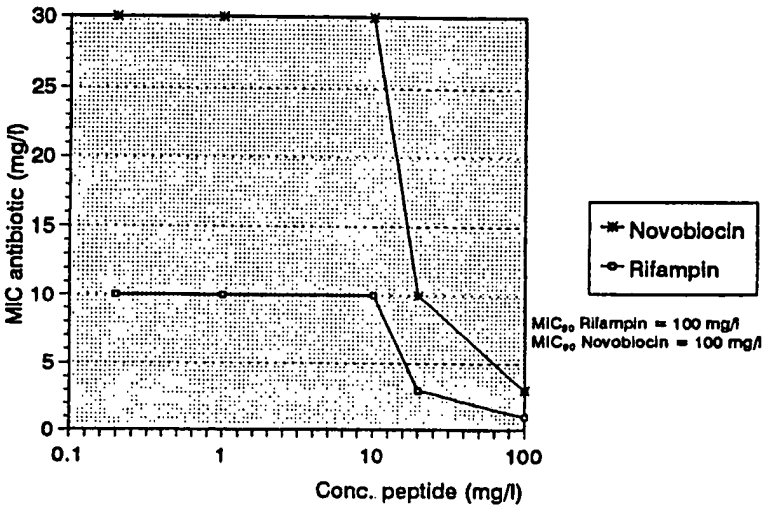


Fig.2c



INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 96/02313

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/04 A61K45/06 //(A61K38/04,31:71),(A61K38/04,31:575), (A61K38/04,31:495)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE (WASHINGTON D C), 259 (5093). 1993. 361-365., XP000608499 RUSTICI A ET AL: "MOLECULAR MAPPING AND DETOXIFICATION OF THE LIPID A BINDING SITE BY SYNTHETIC PEPTIDES" see page 361, column 2, paragraph 2 - page 363, column 1, paragraph 1 ---	1-57
Y	WO,A,90 12587 (MAGAININ SCIENCES INC) 1 November 1990 see page 1, paragraph 2 - page 2, paragraph 1 -----	1-57

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 November 1996	15. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 96/02313

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-49,52,54,56
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claims searched incompletely: 1-57
In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9012587	01-11-90	US-A- 5254535	19-10-93
		AT-T- 135230	15-03-96
		AU-A- 5439490	16-11-90
		CA-A- 2013738	17-10-90
		CN-A- 1046462	31-10-90
		DE-D- 69025927	18-04-96
		DE-T- 69025927	01-08-96
		EP-A- 0469036	05-02-92
		JP-T- 4507087	10-12-92